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The consumption of viruses returns energy to food chains

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Viruses impact host cells and have indirect effects on ecosystem processes. Plankton such as ciliates can reduce the abundance of virions in water, but whether virus consumption translates into demographic consequences for the grazers is unknown. Here, we show that small protists not only can consume viruses they also can grow and divide given only viruses to eat. Moreover, the ciliate *Halteria* sp. foraging on chloroviruses displays dynamics and interaction parameters that are similar to other microbial trophic interactions. These results suggest that the effect of viruses on ecosystems extends beyond (and in contrast to) the viral shunt by redirecting energy up food chains.

virovory | viral shunt | microbial loop

Many known viruses cause diseases, and consequently, virology has long focused on viruses as pathogens. Viruses also affect ecosystem processes, however, by lysing microbes and causing the release of nutrients (i.e., the viral shunt) and through the indirect consequences of host mortality (1, 2). Both of these research domains place viruses as the top "predator" in their food chains, but like most predators, viruses also can serve as food.

Many foragers that swallow water, soil particles, or leaves routinely ingest virus particles. Given the small mass of virus particles relative to other foods, the consumption of viruses is thought to be calorically unimportant (3, 4) and not of sufficient magnitude to influence ecosystem processes. Nonetheless, viruses contain amino acids, nucleic acids, and lipids (5), and if consumed in sufficient quantities could influence the population dynamics of the species that consume them. Some ciliates and flagellates may ingest many viruses (3, 4, 6, 7), but the demographic impact of virus consumption (virovory) is unclear.

Here, we investigate the potential for virovory to fuel population growth and alter the pathways of energy flow in food webs. We measured the population growth of *Halteria* sp. and *Paramecium bursaria* in foraging trials with and without supplemental chloroviruses. We also tracked the reduction in chloroviruses and fit a classic trophic link model to the data to determine whether the *Halteria*–chlorovirus interaction can be viewed as a trophic interaction. Finally, we used fluorescent microscopy to confirm the ingestion of chloroviruses by ciliates.

Results

We found that both *Halteria* and *Paramecium* reduced chlorovirus plaque-forming units (PFUs) by up to two orders of magnitude in 2 d (Fig. 1 *A* and *B*). Fluorescent images showed chloroviruses in vacuoles (Fig. 1 *E*–*H*). The PFU density reduction and images together confirm a large flow of energy and matter from virus populations to a consumer. Moreover, our foraging trials demonstrated robust growth in the *Halteria* population with only chloroviruses as food (r_{int} = 0.66 ± 0.26 [SD], black lines, Fig. 1*A*), with minimal to no growth in the controls (with chloroviruses filtered out; r_{int} = 0.22 ± 0.12 [SD], blue lines, Fig. 1*C*). The abundance of the larger *Paramecium* did not increase in treatment or control trials (Fig. 1*D*), indicating that not all ciliates can grow on chloroviruses in these conditions, even when they consume them.

Dynamics of *Halteria* and chlorovirus abundances fit well to a trophic interaction model (Fig. 2 *A* and *B*) that also provides estimates of key interaction parameters (*SI Appendix*). For space clearance rate (a), the estimate was 0.20 mL per predator per day (CIs: 0.11 to 0.30), which is in the realm of expected values for other foraging protists (8). For conversion efficiency (e), the estimate was 4.6×10^{-7} (CIs: 3.1×10^{-7} to 6.3×10^{-7}). After accounting for biovolume (*SI Appendix*), this translates to a gross growth efficiency of 17%, which compares well with estimates for other aquatic consumers (means are typically 10 to 30%) (9).

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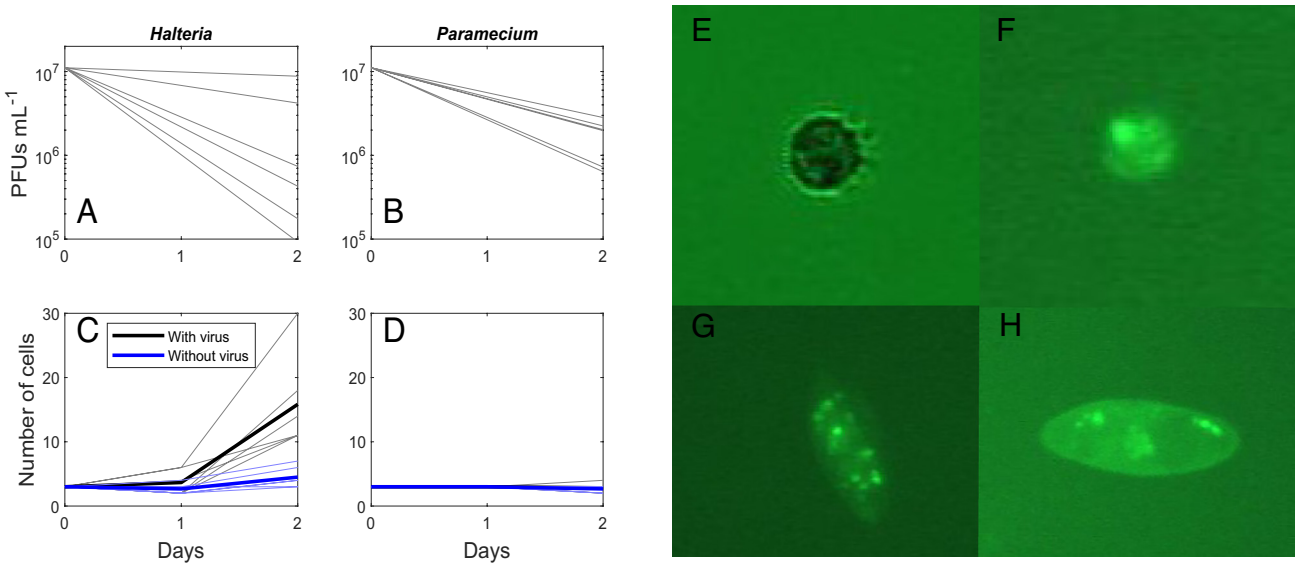

Fig. 1. *Halteria* (*A*) and *Paramecium bursaria* (*B*) reduced plaque-forming unit density by twofold to two orders of magnitude in 2 d. Supplemental feeding of chloroviruses to the ciliate *Halteria* led to pronounced growth (*C*, black lines); in control dishes without virus, *Halteria* cell abundance was steady (*C*, blue lines). *Paramecium* (*D*) showed no cell growth in response to feeding with viruses. Light solid lines are individual replicates; thick lines are averages. Fluorescent micrographs of ciliates fed chloroviruses show *Halteria* with visible light (E) and with fluorescently labeled virus given as food (F). Two other ciliates, *Euplotes sp.* (*G*) and *Paramecium caudatum*(H), also show the indication of virus uptake. Aggregations of viruses are visible in the inside of vacuoles.

Discussion

Our results show that some ciliates can consume enough virus particles to foster population growth at a level similar to protist growth generally (10). Small protists are themselves consumed by zooplankton, so this viral-derived energy and matter may move up through aquatic food webs, altering their structure and dynamics. Protists represent a large fraction of living biomass (11), and their grazing plays a major role in aquatic food webs (12), but current models of aquatic food webs and ecosystems do not include the trophic link between viruses and their consumers. Thus, current food web models are missing a critical interaction, echoing previous work demonstrating the importance of multicellular parasites in food webs more generally (13, 14).

Given the abundance of virus particles in water (1), the abundance of small aquatic protists, and the amount of water in the photic zone globally, the consumption of virus particles by protists could represent a significant and globally relevant trophic transfer. We estimate that each *Halteria* in our experiment ate ~10^4 to 10^6 viruses per day, suggesting that 10^{14} to 10^{16} virions could be consumed per day in a small pond (*SI Appendix*). The viral shunt is thought to limit the movement of energy up food webs by short-circuiting the grazer–microbe foraging interaction (1, 2). Our results indicate, however, that energy and nutrients from host microbes may pass through viruses into grazers and move up the food chain through virovory. This flow would depend on virion size and nutritional content, which varies among strains (15), but it is already clear that viruses of a wide range of sizes can be taken up by grazing protists (3, 4, 6, 7).

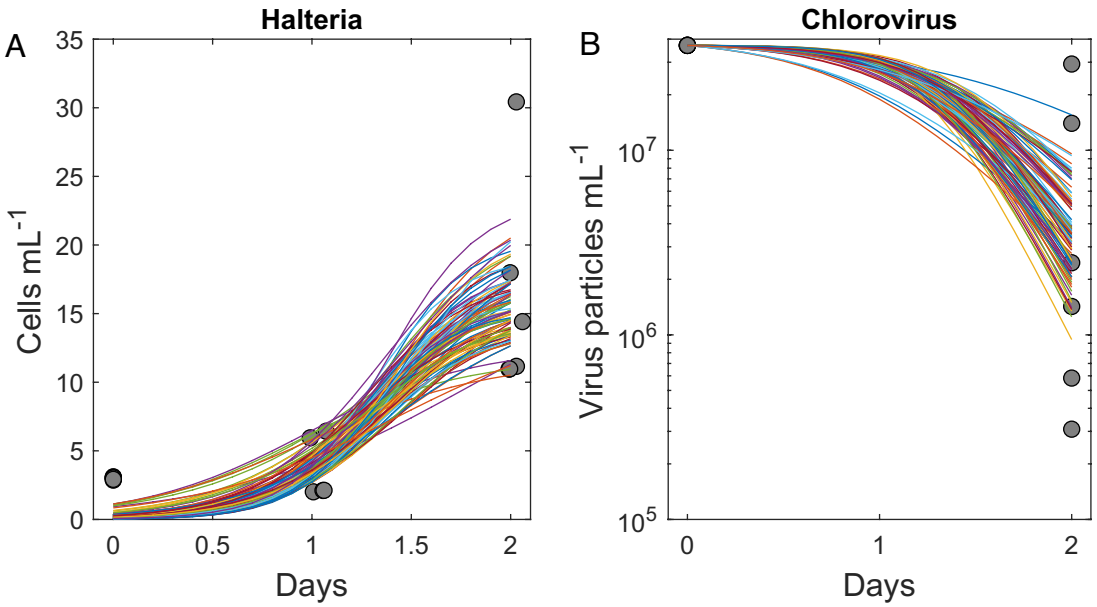

Fig. 2. Predator–prey dynamics of *Halteria* foraging on chloroviruses. *Halteria* grew in abundance (*A*) as it decreased the abundance of chloroviruses (*B*). Individual colored lines are separate bootstrapped model fits.

Our results suggest that virion persistence in the environment depends not only on environmental factors (16) but also on grazing by predators. It is therefore possible that grazers exert selective pressure and influence the evolution of viral phenotypes (17) in a way that interacts with pressure on viruses to effectively infect and replicate within hosts. At this point, however, the evolutionary effects of grazing on viruses are unknown.

Materials and Methods

We conducted foraging trials with *Halteria* and *Paramecium* as grazers. We created 0.4 mL foraging arenas on a 100-mm Petri dish lid and applied two treatments. In the virus treatment, we added 0.5 mL washed virus (2×10^7 PFUs per mL) to the drop with ciliates. In the control treatment, we added 0.5 mL of the virus concentrate cleared of virus with a 0.1-μm syringe filter. We conducted six replicate treatment and control trials at 22 °C. We counted ciliates after 1 and 2 d, and we then used the plaque assay to enumerate infectious chlorovirus particles for the initial virus preparation, the initial rinsed ciliate stocks, and the virus treatment drops at the end of the experiment.

We stained chloroviruses overnight at 6 °C with SYBR Green and washed the virus 10×. We used a Lumascope 400 (San Diego, CA) inverted microscope to image cells at 20×. We tested for uptake in *Halteria* and two other common ciliates, *P. caudatum* and *Euplotes* sp.

To determine whether the virus and *Halteria* dynamics were consistent with a trophic interaction, we fit a trophic link model to the data (*SI Appendix*). We used the PottersWheel Toolbox in MATLAB 2021a to fit the model to 100 bootstrapped datasets and then used the median of the fitted parameters as an estimate of the system-level parameters.

Data, Materials, and Software Availability. Experimental data for ciliate growth and virus consumption, images, and analysis code are hosted on the Zenodo public repository at https://doi.org/10.5281/zenodo.7410482 (18).

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